

Attorney Docket No.: PENN-0708
Inventors: Greene et al.
Serial No.: 09/624,946
Filing Date: July 25, 2000
Page 2

In the Specification

At page 1, line 1, please replace the title with the following amended title:

✓ --METHODS, SYSTEMS AND KITS FOR IMMUNO-DETECTION OF
EPITOPES EXPRESSED ON MOLECULES--

Paragraph beginning at line 17 of page 2 has been amended as follows:

a Suzuki et al. (Jpn. J. Cancer Res. 1995 86:885-89) describe a method called double determinant immuno-polymerase chain reaction (double-determinant immuno-PCR) which utilizes two monoclonal antibodies, in which the antigens are sandwiched, and a specific DNA molecule is used as a marker. In this method, the first monoclonal antibody to bind the circulating antigen is immobilized instead of the antigen itself. A biotinylated second monoclonal antibody is bound to the antigen and free streptavidin is used to attach a biotinylated DNA to the second monoclonal antibody. The biotinylated DNA complexed with antigen-antibody-streptavidin is amplified by PCR. The products are then analyzed by Southern blot analysis.

Attorney Docket No.: PENN-0708
Inventors: Greene et al.
Serial No.: 09/624,946
Filing Date: July 25, 2000
Page 3

Paragraph beginning at line 4 of page 6 has been amended as follows:

a²
Fv fragments for selected epitopes can be produced in cells or on microorganisms by use of recombinant DNA technology. For example, Skerra and Pluckthun (Science 1988 240:1038-1041) describe an expression system for production of functional Fv fragments in *E. coli*.

Paragraph beginning at line 19 of page 9 has been amended as follows:

a³
In one embodiment, the site on the Fv or CDR to which the oligonucleotides are attached comprises a series of residues which allow the attachment of linkers consisting of chemicals such as heterodimeric coupling reagents or other linkers. These residues provide a uniform binding site for the linker attachment. The linkers attach to this site and also links oligonucleotides to the Fv or CDR. Oligonucleotides may be unmodified or modified. For example, the presence of the amplified oligonucleotide can be enhanced by incorporating a beacon or fluorescent labeled oligonucleotide into the mixture allowing for rapid semi quantitative assessment of the epitope expressing molecules (Tan et al. Chemistry 2000 6:1107-1111; Leone et al. Nucleic Acids Res. 1998 26(9):2150-2155).

Attorney Docket No.: PENN-0708
Inventors: Greene et al.
Serial No.: 09/624,946
Filing Date: July 25, 2000
Page 4

Paragraph beginning at line 17 of page 12 has been amended as follows:

a4
The method of the present invention is also useful in the detection of post-translation modifications. PCR and aRNA techniques were originally developed to detect the activity of target genes at the DNA level. These methods have been adopted exclusively in the application of genomics research, sometimes combined with hybridization. Regardless of sensitivity, these methods are not able to detect the post-translation modification at the protein level. Monitoring of such events, however, is very critical since many modifications including, but not limited to, phosphorylation and glycosylation are related to the functional status of the protein. Thus, experiments were performed to demonstrate the ability of the method of the present invention to detect the phosphorylation of the p185 receptor induced by EGF treatment. A signaling model was established in which, upon EGF stimulation, EGFR heterodimerizes with and trans-activates p185, resulting in the phosphorylation of tyrosine residues on the p185 receptor (Qian et al. Proc. Natl Acad. Sci. 1994 91:1500-1504). The A431 cell line, which over expresses EGFR as well as p185 erbB2, was used in these experiments. After EGF stimulation, the p185 receptor in the cell lysate was captured by 1E1, a monoclonal

Attorney Docket No.: PENN-0708
Inventors: Greene et al.
Serial No.: 09/624,946
Filing Date: July 25, 2000
Page 5

antibody developed against p185erB2/neu. PY99, an IgG2b type of anti-phosphorylated Tyr antibody, was used to detect phosphorylated receptors. A second antibody, anti-IgG2b, coupled with ds-oligo, was used to probe the antigen-antibody sandwich complex. A431 cells stimulated with EGF produced a positive band, which was not observed in cells without EGF treatment. T6-17 cells, however, also showed a positive band, indicating constitutive phospho-tyrosine on the p185 receptor. These data indicate that this method is capable of detecting the functional status of a protein by analyzing its modification. Epitope detectors comprising an Fv or CDR coupled to the ds-oligo can also be used to detect the functional status of the protein.

Paragraph beginning at line 25 has been amended as follows:

The original immuno-PCR used pure antigens in the assay. Later iterations of Immuno-PCR examined mixed antigens (Hendrickson et al. Nucleic Acids Research 1995 23(3):522-529) but only showed sensitivity of two to three orders of magnitude higher than ELISA. In a real-world assay with the background comprising a huge variety of non-specific antigens, sensitivity is always limited by the specificity of the assay. Epitopes bound by the Fvs or CDR fragments are expected to identify larger polypeptides and can be used to identify motifs in supernatants, fluids, extracts of cells

Attorney Docket No.: PENN-0708
Inventors: Greene et al.
Serial No.: 09/624,946
Filing Date: July 25, 2000
Page 6

or bacteria or any other eukaryotic organism. Further, actual identity of the polypeptides, organic molecules or sugar structures can be determined by computer aided analysis of data bases using the binding of several epitopes by Fvs as a guide. For example, binding by Fv a, d, e, and f would identify a sugar molecule as having side chains a, d, e, and f, and hence belonging to a family of sugars having these same side chains. In this way the present invention allows definition and identification of many, if not all molecules in a cell at any one particular time. Moreover this approach can be used to identify alternative transcriptional forms translated in an active cell or cellular supernatant. This procedure is easily amenable to 1) use with nonradioactive detection methods, 2) microtized liquid handling procedures, 3) low sample volume detection such as "protein chip" analysis and 4) robotization.
